Remarks

Applicants acknowledge the provisional rejection of Claims 11 and 12 over Application Nos. 09/436,265; 09/939,483; 09/939,484; and 09/892,360. Applicants respectfully request that further treatment of the rejection be held in abeyance. Support for the Applicants' amendment to Claim 11 can be found on page 6, line 17, to page 9, line 2, and Figs. 1 and 2. In light of the Examiner's helpful comments, the Applicants submit new Claim 27, which is drawn to the subject matter of the elected invention. No new matter has been added.

Claim Rejections – 35 U.S. C. § 101

Claims 11 and 12 have been rejected under 35 U.S.C. § 101. Applicants have canceled Claim 12. Applicants respectfully submit that the claimed potassium channel protein (TWIK) as described in the Specification inherently possesses substantial and credible utility. Applicants respectfully submit that the current Office Action statement that "they have not disclosed the specific functions of this protein which separate it from other known potassium channel proteins," is more analogous to an obviousness-type rejection than a utility rejection.

The Office Action apparently applies Example 12 of the Utility Examination Guidelines as a model for the utility rejection. Applicants, however, respectfully submit that Example 12 is inapplicable to the current application. Rather, Applicants respectfully submit that the current application is more analogous to Example 10 of the Utility Guidelines. (Copy enclosed). Specifically, the Applicants have disclosed a particular sequence identification number, which encodes the potassium channel protein. Similarly, Example 10 reveals a SEQ ID No. which encodes

a DNA ligase. Example 10 further reveals that there were a number of DNA ligases known at the time of the filing of the hypothetical Example 10. Nowhere in Example 10 is it recognized that the DNA ligase disclosed in Example 10 needs to be distinguishable from the DNA ligases known in the art to overcome a utility rejection. The Applicants submit that there are over 80 potassium channels known in the art, which demonstrate a wide range of physiological roles within various organisms.

Moreover, the analysis of Example 10 states that DNA ligases have a well established use in the molecular biology art, based on the protein's abilities to ligate to DNA. As a result, there is a "well established utility." In the current Office Action, the Examiner has agreed with the Applicants' assertion that TWIK acts as a potassium channel and thus plays a role in the physiological function associated with variations of electrical potentials. In view of this fact, the Applicants submit that there is already "a well-established utility" known in the art for potassium channels. This understanding, when taken with the identification of the specific sequence identifier, would cause a skilled artisan to recognize the Applicants' potassium channel as having a specific, substantial and credible utility based on its role as a transport mechanism in the creation of electrical potentials within an organism.

While it is true that a later dated publication cannot supplement an insufficient disclosure, a later dated publication may be offered as evidence of the level of ordinary skilled in the art at the time of the application and as evidence that the **disclosed device would have been operative**. Gould v. Quigg, 3 USPQ2d 1302, 1305 (CAFC 1987). Consequently, the Applicants had previously submitted the paper by Wildemann et al., to show that the Applicants' TWIK protein is operative

in the nervous system pathology, and consequently, can be used for treatment and identification of associated nervous system disorders.

Turning now to the consideration of the utility of TWIK protein, the Applicants respectfully submit that the Specification demonstrates that TWIK is a weak inwardly rectifying potassium channel as described on page 3 of the Applicants' Specification. Considering the knowledge of weak inward rectification potassium channels, the utility of TWIK would be well recognized by those skilled in the art. As an example, the identification of a TWIK deficiency, once identified, would be useful to observe a pathological effect exhibited by the resulting TWIK deficiency. Wildemann et al. used the physiological functions of TWIK proteins to identify transport deficiencies and to describe the nervous system pathology.

The Court in Nelson v. Bowler held that the identification of a specific pharmacological activity provides an immediate benefit and therefore satisfies the requirement of 35 U.S.C. § 101. Nelson v. Bowler, 206 USPQ 881, 883 (CCPA 1980). Courts have continuously recognized that when the prior art discloses a compound having a proven action, then a structurally similar compound may inherently possess the same utility. In re Brana, 34 USPQ2d 1436, 1442 (CAFC 1995). Moreover, it is well-established principle of law that pharmaceutical related inventions necessarily include the expectation of further research and development. Nelson, 206 USPQ at 883. The Court in Nelson v. Bowler stated that:

> Knowledge of the pharmacological activity of any compound is obviously beneficial to the public. It is inherently faster and easier to compact illness and alleviate symptoms when the medical profession is armed with an arsenal of chemicals having known pharmacological activities. Since it is crucial to provide researchers with an incentive to disclose pharmacological activities in as many compounds as

> possible, we conclude that adequate proof of any such activity constitutes a showing of practical utility.

The holding in Nelson v. Bower is applicable to this case. The Applicants have identified a particular compound, namely protein channels contained within organisms, which are altered by the addition of, for example, quinine (an alkaloid extract from the bark of the cinchona tree which is used to treat malaria and fever). (See page 9 of the Specification). The Applicants have also demonstrated that the drug dinitrophenol (a metabolic inhibitor that decouples the H^+ gradient in mitochondria and induces internal acidity) induces the inhibition of currents across the TWIK channel. (See page 11 of the Specification). Consequently, the Applicants have clearly demonstrated the pharmacological action of two separate drugs on TWIK channels. Learning how to control potassium channel currents in an organism provides an invaluable tool in both diagnostic and treatment of conditions associated with channel disorders. For the Examiner's convenience we have also enclosed herewith an article entitle "A functional role for the two-pore domain potassiumchannel TASK-1 in cerebellar granule neurons" by Millar et al. This article articulates the functional role of TASK-1, a member of the 2 pore domain K^+ family, in the central nervous system.

The Applicants also respectfully invite the Examiner's attention to page 15, wherein it is stated that "the gene of the TWIK channel has been located on chromosome 1 at position q42-q43." Consequently, the chromosomal location of this gene can act as a determinant for the identification of genetic diseases associated with TWIK potassium channel proteins.

It is respectfully submitted that the Applicants have identified naturally occurring proteins and DNA sequences which are known to be expressed in organisms. Logically, this organism would not produce the protein or express the DNA sequence unless it were advantageous to the organism. One

skilled in the art would therefore recognize that the protein was inherently useful to the organism and as a result necessarily demonstrates inherent utility. The law has recognized that inherent utility or a utility which is obvious to persons skilled in the art "need not be specifically stated." Standard Oil v. Montedison, 212 USPQ 327, 343 (3d Cir. 1981).

The knowledge of the TWIK 1 structure allows the prenatal diagnosis of associated genetic diseases, and the potential for gene therapy.

Claim Rejections Under 35 U.S.C. § 112

Claim 11 has been rejected under 35 U.S.C. § 112, second paragraph. In accordance with the Examiner's helpful suggestion, the Applicants have amended Claim 11 to recite the full phrase referring to the acronym TWIK 1. Specifically, the phrase "tandem of P domain in a weak inward rectifying potassium channel," which stands for TWIK-1 is illustrated on page 3 of the Applicants' Specification. As a result, Applicants respectfully submit that the rejection under 35 U.S.C. § 112, second paragraph, is now obviated. No new matter has been added.

Claims 11 and 12 have been rejected under 35 U.S.C. § 112, first paragraph. Applicants have amended Claims 11 to further point out the specific structural and functional aspects of the TWIK-1 family of proteins. In this respect, the Applicants have identified that TWIK-1 comprises SEQ. ID. NO. 2, and functionally equivalent derivatives, which have clearly distinguishable structural features which would allow one skilled in the art to identify those sequences which were functional equivalents to SEQ. ID. NO. 2. Withdrawal of the rejection of Claim 11 under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Claims Rejections Under 35 U.S.C. § 102

Claim 11 has been rejected under 35 U.S.C. 102(b) as being anticipated by Ketchum et al. As was frankly acknowledged in the Office Action, Ketchum et al discloses a protein having eight transmembrane domains. Applicants amended Claim 11 includes SEQ ID. NO 2, and the functional derivatives of SEQ ID. NO. 2, which consist of two pore domains and four transmembrane domains. Consequently, Ketchum et al fails to disclose a protein comprising SEQ. ID. NO. 2, or the functional equivalents thereof. Withdrawal of the rejection under 35 U.S.C. §102 is respectfully requested.

In view of the foregoing, Applicants respectfully submit that the application is now in condition for allowance, which action is respectfully requested.

Respectfully submitted,

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REVISED INTERIM UTILITY GUIDELINES TRAINING MATERIALS

Contents

characterize the protein. A starting material that can only be used to produce a final product does not have a substantial asserted utility in those instances where the final product is not supported by a specific and substantial utility. In this case none of the proteins that are to be produced as final products resulting from processes involving the claimed cDNA have asserted or identified specific and substantial utilities. The research contemplated by Applicants to characterize potential protein products, especially their biological activities, does not constitute a specific and substantial utility. Identifying and studying the properties of the protein itself or the mechanisms in which the protein is involved does not define a "real world" context of use. Note, because the claimed invention is not supported by a specific and substantial asserted utility for the reasons set forth above, credibility has not been assessed. Neither the specification as filed nor any art of record discloses or suggests any property or activity for the cDNA compounds such that another non-asserted utility would be well established for the compounds.

Claim 1 is also rejected under 35 U.S.C. § 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention.

Example 10: <u>DNA Fragment encoding a Full Open Reading Frame (ORF)</u>

Specification: The specification discloses that a cDNA library was prepared from human kidney epithelial cells and 5000 members of this library were

sequenced and open reading frames were identified. The specification discloses a Table that indicates that one member of the library having SEQ ID NO: 2 has a high level of homology to a DNA ligase. The specification teaches that this complete ORF (SEQ ID NO: 2) encodes SEQ ID NO: 3. An alignment of SEQ ID NO: 3 with known amino acid sequences of DNA ligases indicates that there is a high level of sequence conservation between the various known ligases. The overall level of sequence similarity between SEQ ID NO: 3 and the consensus sequence of the known DNA ligases that are presented in the specification reveals a similarity score of 95%. A search of the prior art confirms that SEQ ID NO: 2 has high homology to DNA Ligase encoding nucleic acids and that the next highest level of homology is to alpha-actin. However, the latter homology is only 50%. Based on the sequence homologies, the specification asserts that SEQ ID NO: 2 encodes a DNA ligase.

Claim 1: An isolated and purified nucleic acid comprising SEQ ID NO: 2.

Analysis: The following analysis includes the questions that need to be asked according to the guidelines and the answers to those questions based on the above facts:

1) Based on the record, is there a "well established utility" for the claimed invention? Based upon applicant's disclosure and the results of the PTO search, there is no reason to doubt the assertion that SEQ ID NO: 2 encodes a DNA ligase. Further, DNA ligases have a well-established use in the molecular biology art based on this class of protein's ability to ligate DNA. Consequently the answer to the question is yes.

Note that if there is a well-established utility already associated with the claimed invention, the utility need not be asserted in the specification as filed. In order to determine whether the claimed invention has a well-established utility the examiner must determine that the invention has a specific, substantial and credible utility that would have been readily apparent to one of skill in the art. In this case SEQ ID NO: 2 was shown to encode a DNA ligase that the artisan would have recognized as having a specific, substantial and credible utility based on its enzymatic activity.

Thus, the conclusion reached from this analysis is that a 35 U.S.C. § 101 rejection and a 35 U.S.C. § 112, first paragraph, utility rejection should not be made.

Example 11: <u>Animals with Uncharacterized Human Genes</u>

Specification: Kidney cells from a patient with Polycystic Kidney (PCK) Disease have been used to make a cDNA library. From this library 8000 nucleotide "fragments" have been sequenced but not yet used to express proteins in a transformed host cell nor have they been characterized in any other way. The 50 longest fragments, SEQ ID NO: 1-50, respectively, have been used to make transgenic mice. None of the 50 lines of mice have developed Polycystic Kidney Disease to date. The asserted utility is the use of the mice to research human genes from diseased human kidneys. The disease is inheritable, but chromosomal loci have not yet been identified. Neither the absence or presence of a specific protein has been identified with the disease condition.

A functional role for the two-pore domain potassium channel TASK-1 in cerebellar granule neurons

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Cerebellar granule neurons (CGNs) are one of the most populous cells in the mammalian brain. They express an outwardly rectifying potassium current, termed a "standing-outward" K^+ current, or IK_{SO}, which does not inactivate. It is active at the resting potential of CGNs, and blocking IK_{SO} leads to cell depolarization. IK_{SO} is blocked by Ba^{2+} ions and is regulated by activation of muscarinic M_3 receptors, but it is insensitive to the classical broad-spectrum potassium channel blocking drugs 4-aminopyridine and tetraethylammonium ions. The molecular nature of this important current has yet to be established, but in this study, we provide strong evidence to suggest that IK_{SO} is the functional correlate of the recently identified two-pore domain potassium channel TASK-1. We show that IK_{SO} has no threshold for activation by voltage and that it is blocked by small extracellular acidifications. Both of these are properties that are diagnostic of TASK-1 channels. In addition, we show that TASK-1 currents expressed in *Xenopus* oocytes are inhibited after activation of endogenous M_3 muscarinic receptors. Finally, we demonstrate that mRNA for TASK-1 is found in CGNs and that TASK-1 protein is expressed in CGN membranes. This description of a functional two-pore domain potassium channel in the mammalian central nervous system indicates its physiological importance in controlling cell excitability and how agents that modify its activity, such as agonists at G protein-coupled receptors and hydrogen ions, can profoundly alter both the neuron's resting potential and its excitability.

On the basis of sequence similarities, the pore-forming α-subunits of K channels have been grouped into superfamilies. The two principal superfamilies are the inward rectifier or 2TM (for two transmembrane domains) superfamily and the voltage-gated or 6TM superfamily. Most electrophysiologically characterized native K channels are encoded by genes from one or the other of these two superfamilies, although in most cases, the exact subunit configuration that underlies each native current is not clear (1–3). During the last 4 years, a third major superfamily of K channels, the two-pore domain potassium channel family (2-PK) (4), has emerged, initially identified from yeast K channel sequencing (4, 5) and now identified in mammalian cells (6–14).

In mammals, six functional members of the 2-PK family with 4TM domains (or TWIK family) have been identified to date. These are TWIK-1, TWIK-2, TREK-1, TASK-1, TASK-2, and TRAAK (6–14). Despite a relatively low sequence similarity and different functional properties, these 2-PK channels all produce quasiinstantaneous and noninactivating currents (although TASK-2 currents have relatively slow activation kinetics; ref. 13). The 2-PK channels are presently classified into three distinct functional subfamilies (13). TASK-1 and TASK-2 are sensitive to small variations in external pH close to physiological pH; only TASK-1 is expressed in the nervous system to any significant degree (6, 9, 13). TREK-1 and TRAAK are arachidonic acid-activated, mechanosensitive K channels (7, 8, 11, 12), whereas TWIK-1 and TWIK-2 are weakly inward rectifying K channels (10, 14). To date, no functional correlates of 2-PK channels have been identified in the mammalian nervous system.

Cerebellar granule neurons (CGNs) express an outwardly rectifying potassium current, termed a "standing-outward" K^+ current or IK_{SO}, (15). IK_{SO} does not inactivate and is active at the resting potential of CGNs; blocking IK_{SO} leads to cell depolarization. IK_{SO} is insensitive to the classical broad-spectrum potassium channel blockers 4-aminopyridine and tetraethylammonium, but it is blocked by Ba^{2+} ions and regulated by activation of G protein coupled receptors, such as muscarinic M_3 receptors. In this study, we provide evidence to suggest that IK_{SO} is the functional correlate of the 2-PK channel TASK-1.

Materials and Methods

CGNs. CGNs were isolated from 6- to 9-day-old Sprague–Dawley rats and cultured as described (15), and cerebellar slices (250 μm thick) were prepared from 3- to 5-week-old male mice (TO strain) as described (16).

Oocytes. Oocytes were injected with cRNA transcribed from rTASK-1 cDNA, which was a kind gift from S. Yost (University of California at San Francisco). RNA (2–10 ng) was injected manually into oocytes that were kept for 3 days before recordings.

Electrophysiological Recordings. For cultured cells, currents were recorded in the whole-cell perforated patch-clamp configuration with amphotericin B (240 μg/ml) as the permeabilizing agent from CGNs between 7 and 10 days in culture. Recording solutions have been described (15), and all experiments were performed at room temperature. Solutions were applied by bath perfusion at a rate of 4–5 ml·min^{-1}, and complete exchange of the bath solution occurred within 30–40 s. For slices, perforated patch recordings were made from freshly prepared slices with recording conditions and solutions as described (16). For oocyte recordings, standard two-electrode voltage clamp measurements were performed at room temperature.

Reverse Transcriptase–PCR (RT-PCR). Total RNA was prepared from cell suspensions of CGNs from 10-day-old cultures with the RNeasy miniprep kit (Qiagen, Chatsworth, CA). Samples of RNA were DNase treated and reverse transcribed by using Moloney murine leukemia virus-reverse transcriptase (Promega) and random hexamer primers. PCR was performed with

Abbreviations: CGN, cerebellar granule neuron; 2-PK, two-pore domain potassium; IK_{SO}, standing-outward K^+ current; TM, transmembrane domain; RT-PCR, reverse transcriptase–PCR; GFAP, glial fibrillary acidic protein.

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Taq DNA polymerase (Promega) for 30 cycles. Two sets of primer pairs for TASK-1 were used: 5′-CACCGTCATCACCACAATCG (F1) with 5′-TGCTCTGCATCACGCTTCTC (R1) and 5′-AGTACGTGGCCTTCAGCTTC (F2) with 5′-TGGAGTACTGCAGCTTCTCG (R2). Actin primers were 5′-TTGTAACGAACTGGGACGATATGG with 5′-GATCTTGATCTTCATGGTGCTAGG. PCR products were gel extracted and purified, and their identities were confirmed by sequencing.

Antibody Labeling. Anti-TASK-1, a polyclonal antibody raised in rabbit against a highly purified peptide (TASK 252–269) corresponding to residues 252–269 of human TASK-1 was obtained from both Alomone Labs (Jerusalem) and Chemicon and used at a dilution of 1:100. This epitope is specific for TASK-1 and is highly conserved in mouse and rat TASK-1. Staining was abolished by preabsorption of the antibody with 10 μg/ml of the peptide. Glial cells were labeled in the same CGN cultures with a mixture of mouse monoclonal antibodies against glial fibrillary acidic protein (GFAP; clones 4A11, 1B4, and 2E1; PharMingen; dilution 1:100). Primary antibodies were detected with FITC- and Cy3-labeled secondary antibodies, respectively, and visualized with an Olympus Fluoview laser scanning confocal microscope (New Hyde Park, NY).

Results

It has been suggested that IK_{SO} in CGNs may result from expression of *ether-a-go-go* channels (such as *eag*-1; refs. 17 and 18) or be related to the M current (ref. 19; now thought to be formed from heteromultimers of KCNQ2 and KCNQ3, ref. 20). However, these channels are members of the 6TM family of K channels and have definable thresholds for voltage activation (19, 21). By contrast, a key diagnostic feature of members of the 2-PK channel family is that they are open at all potentials. To help determine the molecular nature of IK_{SO}, we have examined the steady-state characteristics of the current over a range of potentials to determine whether it has a defined activation threshold.

The basic features of IK_{SO} seen in CGNs from both rats and mice are shown in Fig. 1*a*. IK_{SO} can be seen as a noninactivating current when cells are held at −20 mV, which is reduced in amplitude when the cell is hyperpolarized to −60 mV. The current is rapidly and reversibly inhibited by 10 μM muscarine (Fig. 1 *a* and *b*; 68 ± 2%, n = 51) acting on muscarinic acetylcholine receptors. Current-voltage relations were obtained by hyperpolarizing the cell in ramp waveforms as shown in Fig. 1*c*. After step voltage changes, IK_{SO} currents have been shown to reach steady-state with a time constant of 0.5 ms (see ref. 15); therefore, the ramp was sufficiently slow (10 ms/mV) to allow IK_{SO} to reach steady-state at each potential. To ensure that IK_{SO} was studied in isolation, we have exploited the sensitivity to muscarine. The control current-voltage relation for IK_{SO} was obtained by subtracting currents evoked by hyperpolarizing ramps obtained in the presence of muscarine from those in its absence (Fig. 1*e*). The current-voltage relation was outwardly rectifying, and the reversal potential (−90 ± 4 mV, n = 4) was close to the predicted K^+ equilibrium potential (−98 mV) when extracellular [K^+] was 2.5 mM. The protocol was then repeated when the extracellular [K^+] was raised to 25 mM (Fig. 1 *d* and *e*). This process revealed two things. First, the current must be a selective K^+ current, because its reversal potential was shifted in a depolarizing direction to −39 ± 1 mV (n = 4) virtually identical to that predicted by the Nernst equation (−40 mV). Second, IK_{SO} showed no deactivation (did not switch off) over the whole range of potentials tested, and therefore, it has no threshold for activation between these potentials. When [K^+] was raised further to 100 mM (Fig. 1*f*), the reversal potential was shifted further, and the current-voltage relationship linearized, exactly as predicted by the Goldman–Hodgkin–Katz equation and in exactly the way seen for certain cloned 2-PK channels such as TASK-1 (6, 9).

Of the six functional mammalian 2-PK domain channels cloned thus far, only two, TREK-1 and TASK-1, show a number of striking similarities to IK_{SO}. IK_{SO} is insensitive to 4-aminopyridine (10 mM) and tetraethylammonium (5 mM) but blocked by Ba^{2+} (see below), quinidine (100 μM; 43 ± 4%, n = 6), and Na^+ substitution by *N*-methyl-D-glucamine in the external solution (55 ± 4%, n = 7), all of which are features shared by TASK-1 and TREK-1 (6, 7, 9, 12). However, the two main diagnostic features of TASK-1 (in contrast to TREK-1) are its sensitivity to small changes in external pH and its lack of enhancement by arachidonic acid (13).

Treatment of CGNs with arachidonic acid (10 μM) did not enhance IK_{SO}. This result argues against the idea that IK_{SO} current is caused by expression of TREK-1 (12). Indeed, arachidonic acid caused a transient inhibition of IK_{SO} (10.6 ± 2.2%, n = 9), but this inhibition was not maintained (steady-state inhibition was 0.2 ± 1.8%, n = 9), a result consistent with previous observations of TASK-1 (12). Exposure to extracellular solutions of pH 6.4 inhibited IK_{SO} by 77 ± 3% (n = 10) compared with control currents measured at −20 mV in pH 7.4 solution (Fig. 2 *a* and *b*). Changing to an external solution of pH 6.9 gave only 31 ± 4% (n = 4) inhibition, whereas pH 7.9 slightly increased IK_{SO} (7 ± 3%, n = 5) compared with control values. These results are very similar to the effects of changing external pH on TASK-1 currents expressed in *Xenopus* oocytes (6, 9). In our own experiments on heterologously expressed TASK-1 (see Fig. 3*a*), reducing external pH from 7.2 to 6.2 inhibited TASK-1 currents by 72 ± 7% (n = 3), whereas raising it from 7.2 to 8.2 enhanced TASK-1 currents by 25 ± 9% (n = 3). Ramp voltage protocols showed that the degree of inhibition of IK_{SO} by acidification of the external solution was relatively independent of voltage. In current-clamp recordings, exposure to pH 6.4 solution depolarized cells by around 19 mV (from −78 ± 4 to −59 ± 3 mV, n = 5; Fig. 2*c*). The input resistance of CGNs around the resting potential of the cells (−70 to −90 mV) substantially increased from 477 ± 56 MΩ in normal external solution to 1,517 ± 367 MΩ (n = 5) at pH 6.4, indicating that external acidification enhances the excitability of the neurons by block of this resting conductance. Thus, block of IK_{SO} by extracellular acidification will both depolarize CGNs directly and enhance the effects of other depolarizing inputs, such as glutamate acting on ionotropic glutamate receptors.

It is important to determine whether IK_{SO} seen in cultured neonatal CGNs is also present in cells from more mature animals maintained as closely as possible to conditions *in vivo*. Observations on cerebellar slices (250 μm thick) from 3- to 5-week-old mice (where cerebellar synaptogenesis is almost complete; ref. 22) are illustrated in Fig. 3*b*. As with neonatal CGNs in culture (see ref. 15 and Fig. 1), CGNs in acutely isolated slices possess a rapidly activating, noninactivating potassium current with an amplitude of 179 ± 15 pA (n = 41) at −20 mV. As with IK_{SO} in cultured CGNs, IK_{SO} in cerebellar slices is blocked by Ba^{2+} ions; in the slice experiment, the IC_{50} was 0.35 mM (Fig. 3*c*).

The currents resulting from expression of TASK-1 have been studied previously by holding cells at negative potentials and applying step or ramp depolarizations (6, 9). Herein, we show that by holding TASK-1 expressing oocytes at a potential of −20 mV, the currents evoked show no appreciable inactivation over a time scale of seconds between hyperpolarizing steps to −80 mV. Hyperpolarizing steps to −80 mV reduced TASK-1 currents in proportion to the reduction in driving force, exactly as is seen for IK_{SO} in neurons. *Xenopus* oocytes possess endogenous muscarinic receptors thought to be primarily of the M_3 subtype (23, 24). Activation of these receptors by carbachol (100 μM) produced fully reversible 52 ± 6% (n = 4) inhibition of TASK-1

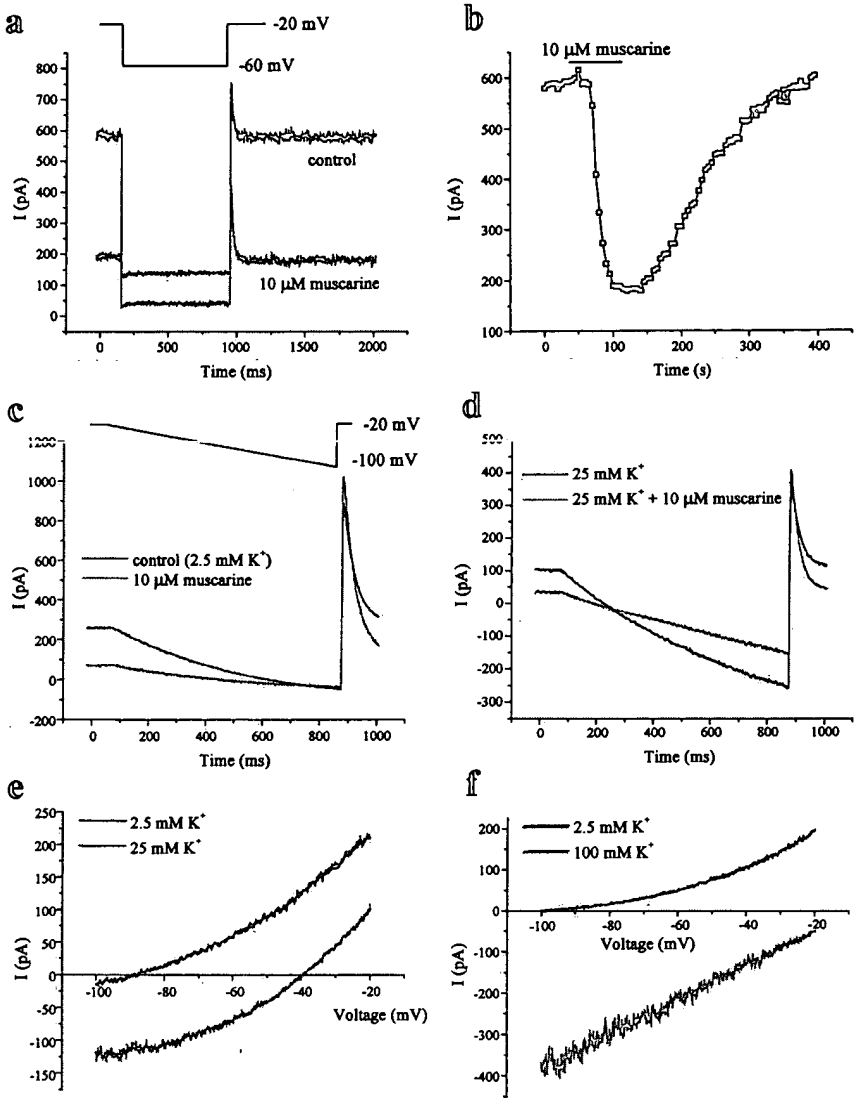

Fig. 1. IK_{SO} in rat cultured CGNs is active at all potentials and is inhibited by muscarine. (*a*) In perforated patch recordings, IK_{SO} is seen as a noninactivating current at −20 mV and is instantaneously reduced in amplitude when cells are stepped to −60 mV for 800 ms once every 6 s. The current is inhibited by muscarine (10 μM). (*b*) Block and washout of block by muscarine is illustrated by measuring the standing-outward current at −20 mV. (*c*) Cells were held at −20 mV and then hyperpolarized to −100 mV by means of a ramp waveform at 0.1 mV/ms over 800 ms once every 6 s in the presence and absence of 10 μM muscarine. (*d*) Cells were treated as described in *c*, except in raised external $[K^+]$ (25 mM). (*e*) The muscarine-sensitive current during ramps is obtained by subtraction and shown in control and 25 mM $[K^+]$. (*f*) Cells were treated as described in *e*, except in control and 100 mM external $[K^+]$.

currents and an associated decrease in membrane conductance (Fig. 3*a*). In addition to their pH and carbachol sensitivity, TASK-1 currents were also inhibited by Ba^{2+} ions with an IC_{50} value of 0.35 mM, identical to the inhibition found for IK_{SO} in CGNs in mouse cerebellar slices (Fig. 3*c*).

RT-PCR experiments with two specific primer pairs for TASK-1 indicated that the mRNA for TASK-1 is present in our CGN cultures (Fig. 4*a*). Furthermore, by using specific anti-TASK-1 antibodies, TASK-1 protein was seen to be expressed in the cytoplasm and surface membrane of CGNs in 7-day-old cultures (Fig. 4*b*). In contrast, negligible labeling for TASK-1 was seen in CGNs cultured for only 1 day, which is consistent with our previous observation of a lack of any measurable IK_{SO} in such cells (15). Double-labeling with antibodies against GFAP indicated that TASK-1 protein was also expressed by glial cells in culture (Fig. 4*b*) and in adult rat astrocytes *in situ* (not shown).

Discussion

In this study, we have shown that the noninactivating K current in CGNs, IK_{SO}, has all the properties predicted for a background K channel belonging to the 2-PK family. Furthermore, its lack of a defined voltage threshold for activation, its regulation by muscarinic receptor activation, and its exquisite sensitivity to changes in the pH of the extracellular solution strongly suggest that IK_{SO} is the functional correlate of the 2-PK channel TASK-1. Earlier *in situ* hybridization studies of Duprat *et al.* (6) showed that TASK-1 mRNA was present in the granule cell layer of the cerebellum. We have extended these observations with RT-PCR and a selective TASK-1 antibody, and we now show that TASK-1 is expressed in CGNs themselves and that the protein is found in the plasma membrane of these cells. Furthermore, protein expression is correlated with the magnitude of IK_{SO}.

The sensitivity of IK_{SO} to small changes in extracellular pH is of particular physiological importance, because extracellular

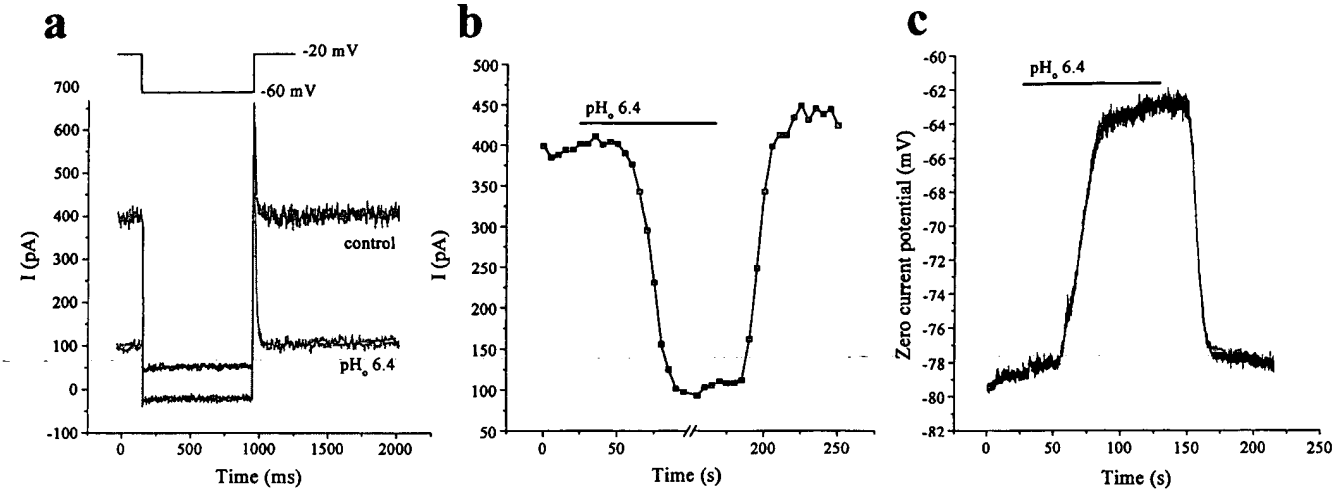

Fig. 2. IK_{SO} in rat cultured CGNs is inhibited by extracellular acidification. (*a*) IK_{SO} current recorded as in Fig. 1*a* is inhibited by changing external pH from 7.4 to 6.4. (*b*) Block and washout of block by pH 6.4 external solution (control pH 7.4) is illustrated by measuring the standing-outward current at −20 mV. (*c*) Current-clamp recordings show depolarization of a CGN after extracellular acidification.

acidification will both depolarize CGNs and increase their excitability after block of IK_{SO}. Substantial decreases in extracellular pH (0.6 units) are known to accompany increases in extracellular glutamate levels with ischemia and are also seen during epileptic seizures (25). Furthermore, transient acid shifts occur during the release of neurotransmitters from storage vesicles (25), which are also highly acidic. These transient acidic shifts are known to facilitate excitatory transmission in the central nervous system (26).

Remarkably, of more than 80 known potassium channel α-subunits identified to date from the sequence of the nematode *Caenorhabditis elegans*, over 50 belong to this emerging family of K channels (27). It is likely, therefore, that the very few 2-PK channels identified thus far in mammalian neurons are the forerunners of a large extended family. It has been suggested recently that they may also represent an important target protein for general anaesthetic agents, which can enhance the activity of certain 2-PK channels thereby decreasing neuronal excitability (28, 29). This functional

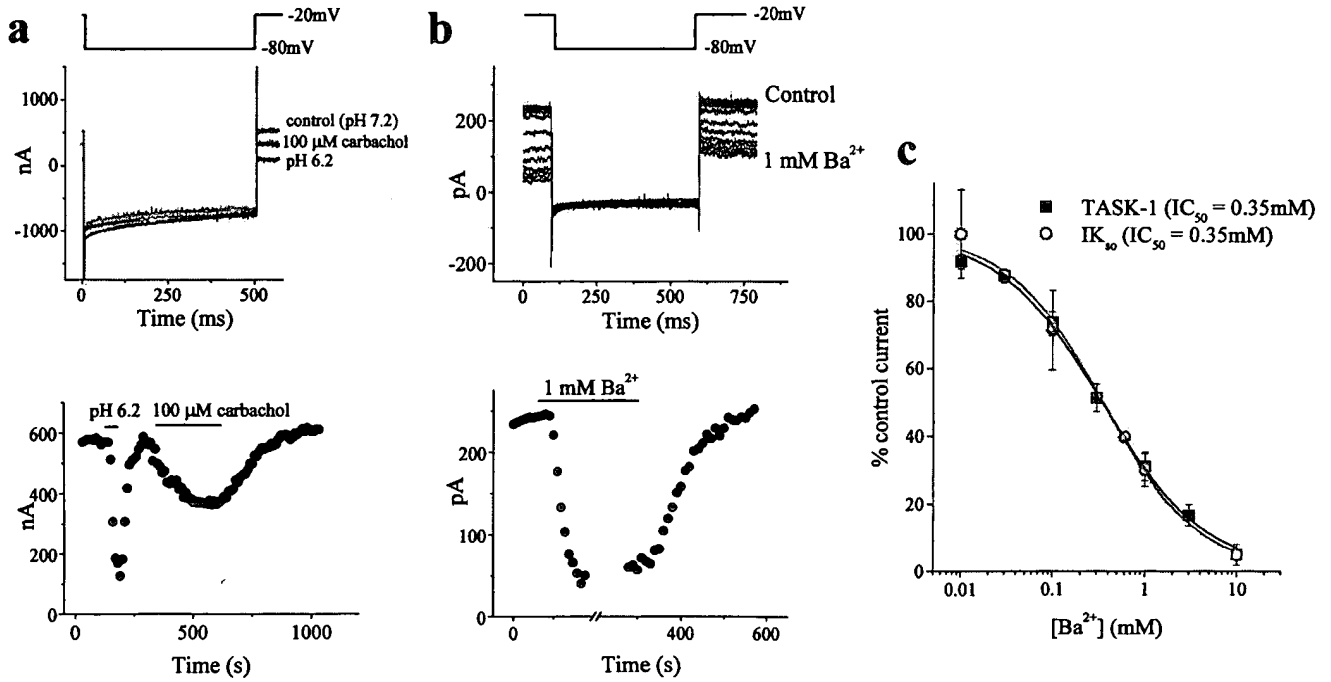

Fig. 3. TASK-1 expressed in oocytes shares properties of IK_{SO} that is present in cerebellar slices. (*a*) TASK-1 currents expressed in oocytes and recorded by stepping to −80 mV for 500 ms once every 10 s from a holding potential of −20 mV have the same current profile as IK_{SO} and are blocked by extracellular acidification and by carbachol. Block by extracellular acidification and carbachol (100 μM) is illustrated (*Lower*) by measuring the standing-outward current at −20 mV. (*b*) IK_{SO}, recorded from CGNs in mouse cerebellar slices, was determined by the same voltage protocol described for *a*. Block by 1 mM Ba^{2+} is illustrated (*Lower*) by measuring the standing-outward current at −20 mV. (*c*) Ba^{2+} sensitivity of TASK-1 in oocytes and IK_{SO} in cerebellar slices.

NEUROBIOLOGY

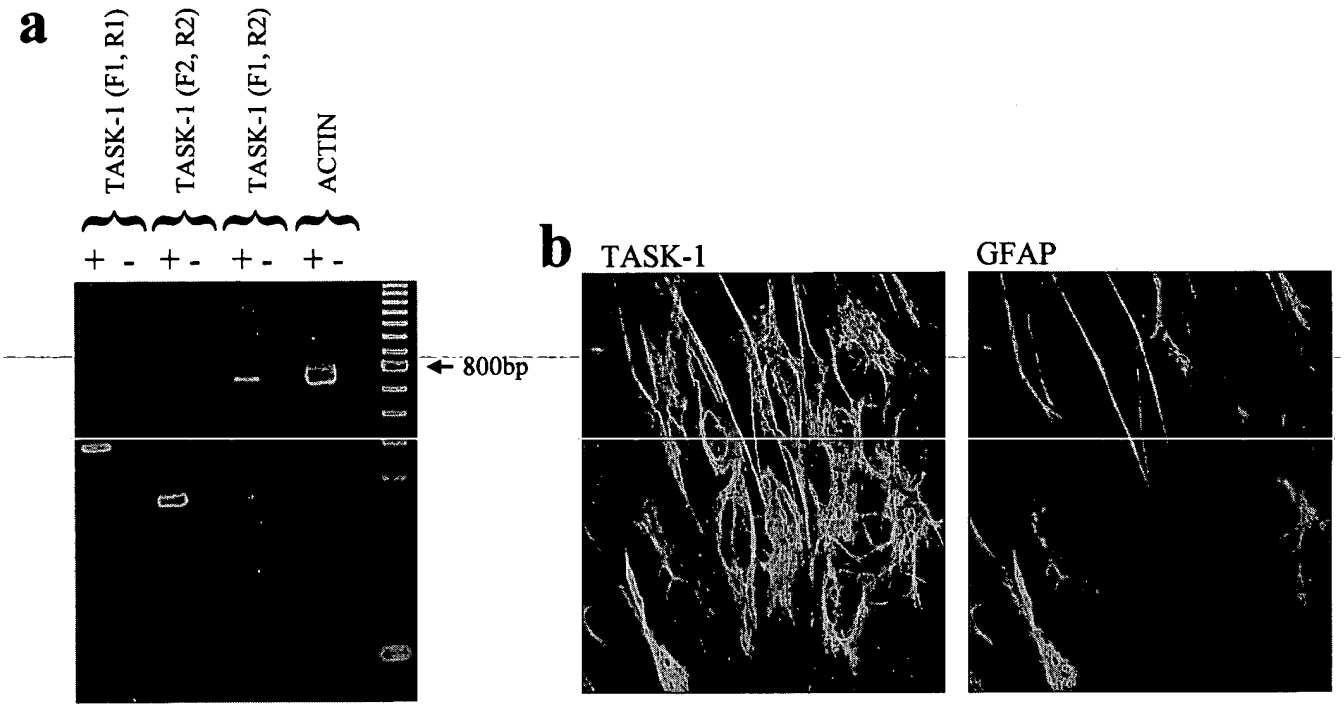

Fig. 4. TASK-1 mRNA is present in CGN cultures and TASK-1 protein is expressed in the membrane of CGNs. (*a*) RT-PCR experiments identify the presence of mRNA for TASK-1 in CGNs cultures. (*b*) TASK-1 antibody (green) and GFAP (red) labeling in CGN cultures in the same field of view. TASK-1 protein appears to be expressed in both the cytoplasm and surface membrane of CGNs. Note also the TASK-1 staining in GFAP-labeled glial cells.

demonstration of a mammalian 2-PK channel in neurons shows the channel's likely importance in controlling cell excitability and also suggests a mechanism for how agents that modify its activity, such as muscarinic receptor agonists and hydrogen ions, can profoundly alter both the neuron's resting potential and its excitability.

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Note Added in Proof. Since the original submission of our work, we have become aware of a study showing functional expression of TASK-1 in hypoglossal motoneurons (30).

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